

Role of Backyard Flocks in Transmission Dynamics of Highly Pathogenic Avian Influenza A(H5N8) Clade 2.3.4.4, France, 2016–2017

Appendix

Methods

Multivariable logistic regression analysis was conducted to investigate risk factors statistically associated with the infection status (AIV and H5). First, associations were tested between each outcome and explanatory variable using a chi-squared test or Fisher's test (Appendix Table 1). Explanatory variables with a p-value below 0.2 were selected to be included in multivariable logistic regressions. Pairwise collinearity was tested between all selected explanatory variables by computing Cohen's kappa coefficient and considered significant if the absolute value of the coefficient exceeded 0.7. For the multivariable regressions, stepwise backward elimination was performed and explanatory variables retained if statistically significant ($p < 0.05$). All analyses were performed using R statistical software (version 3.4.1).

HIT (hemagglutination inhibition tests) were performed following international standards, using different couple of antigens specific for clade 2.3.4.4 H5 viruses, H5N8 A/decoyduck/France/161105a/2016 and H5N5 A/muteswan/ Croatia/102/2016, or for viruses belonging to other H5 Eurasian lineages, H5N3 A/muscovy duck/France/070090b/2007 and H5N2 A/chicken/France/03426a/2003 (<http://www.oie.int/en/standard-setting/terrestrial-code/access-online>). These pairs of H5 antigens, with different neuraminidase subtypes, were tested to exclude cross-reactivity driven by neuraminidase-specific antibodies.

Antigens Used for HIT

H5N2 A/chicken/France/03426a/2003 and H5N3 A/muscovy duck/France/070090b/2007: sera displaying HI titers higher than 16 with both antigens are H5-positive in HIT. The antigens used have a broad cross-reactivity against H5 antibodies induced by infection or immunization with Eurasian lineage H5Nx low pathogenic or HPAI, except H5Nx A/goose/Guandong/1/1996-lineage clade 2.3.4.4 HPAI.

H5N5 A/muteswan/ Croatia/102/2016 (clade 2.3.4.4 A/goose/Guandong/1/1996 lineage) and H5N8 A/decoyduck/France/161105a/2016 (clade 2.3.4.4 A/goose/Guandong/1/1996 lineage): sera displaying HI titers higher than 16 with both antigens are H5-positive in HIT. The antigens used have a narrow cross-reactivity against H5 antibodies induced by infection or immunization with H5Nx A/goose/Guandong/1/1996-lineage clade 2.3.4.4 HPAI.

Appendix Table 1. ELISA, HIT and PCR analyses performed on AIV seropositive backyard poultry.

Positive ELISA									
AIV backyards	Bird ID	Species	ELISA H5	HIT H5N2	HIT H5N3	HIT H5N5	HIT H5N8	PCR M	PCR H5
V5	V5-02	Poultry	-	ND	ND	ND	ND	-	-
V9	V9-01	Duck	+	<4	<2	16	16	-	-
	V9-02	Poultry	+	ND	<2	ND	32	-	-
	V9-03	Duck	±	<4	<2	8	16	-	-
	V9-09	Duck	-	<4	<2	8	8	-	-
	V9-13	Duck	-	<4	<2	<4	<2	-	-
V12	V12-04	Duck	-	ND	ND	ND	ND	-	-
V18	V18-09	Poultry	-	ND	ND	ND	ND	-	-
V21	V21-04	Duck	±	<4	<2	<4	<2	-	-
	V21-06	Poultry	+	ND	ND	ND	ND	-	-
V22	V22-10	Duck	±	<4	<2	<4	<2	-	-
V24	V24-04	Poultry	-	ND	ND	ND	ND	-	-
V32	V32-02	Poultry	-	ND	ND	ND	ND	-	-
V33	V33-02	Poultry	-	ND	ND	ND	ND	-	-
	V33-08	Poultry	-	ND	ND	ND	ND	-	-
	V37	V37-06	Poultry	+	16	32	<4	<2	-
V50	V37-07	Poultry	+	16	16	<4	<2	-	-
	V50-03	Poultry	+	<4	8	<4	<2	-	-
V59	V59-01	Goose	+	32	32	<4	<2	-	-
	V59-02	Goose	+	16	16	<4	<2	-	-
	V59-03	Duck	+	32	16	<4	<2	-	-
	V59-05	Duck	+	<4	<2	<4	<2	-	-
	V59-07	Poultry	+	ND	<2	ND	<2	-	-
	V59-08	Poultry	±	<4	<2	<4	<2	-	-
	V59-09	Poultry	+	<8	16	<4	<2	-	-
	V59-10	Poultry	+	4	8	ND	<2	-	-
V62	V62-03	Goose	-	ND	ND	ND	ND	-	-
V69	V69-01	Duck	+	32	8	<4	<2	-	-
	V69-02	Duck	+	<4	<2	<4	<2	-	-
V78	V78-02	Poultry	-	ND	ND	ND	ND	-	-
	V78-06	Poultry	-	ND	ND	ND	ND	-	-
	V78-09	Poultry	-	ND	ND	ND	ND	-	-
V81	V81-08	Duck	-	<4	<2	<4	<2	-	-
	V81-09	Duck	+	<4	<2	<4	<2	-	-
V87	V87-02	Poultry	-	ND	ND	ND	ND	-	-
V85	V85-03	Poultry	-	ND	ND	ND	ND	-	-

*HIT, hemagglutination inhibition test; ND, Not Done; +, positive results; -, negative results; ±, uncertain results.

Appendix Table 2. Binary variables examined for association with AIV and H5 seropositive holdings and results given by the univariate analysis with $p < 0.05$ * and $p < 0.01$ **.

Variable	Description	Backyard holdings, % (proportion)	p value chi-squared or Fischer test AIV	p value chi- squared or Fischer test H5
Species	Backyard flocks with ducks*	31 (22/70)	0.02*	0.03*
Fenced outdoor	Access to a defined free-range area	76 (53/70)	0.99	0.99
Covered backyard holding	Covered chicken house and/or free-range	19 (13/70)	0.19	0.19
Covered food	Water and food distribution in a covered area	57 (40/70)	0.28	0.46
Change practice	Modification of practices following the first H5 cases in South-Western France	37 (26/70)	0.38	0.70
Clothes	No specific clothing for backyard care	97 (68/70)	0.99	0.99
Shoes	No specific shoes for backyard care	83 (58/70)	0.43	0.71
Handwashing	No handwashing before or after visiting the backyard	33 (23/70)	0.98	0.85
Animal introduction	Bird introduction during the last year	73 (51/70)	0.92	0.70
Backyard holdings' production	Selling or giving eggs to family or neighbors	74 (52/70)	0.51	0.51
Backyard holdings' age	Owners having backyards holdings for more than 30 y	59 (41/70)	0.15	0.33
Bird exhibition visit	Visit of a bird exhibition during the last three months	4 (3/70)	0.99	0.31
Link with poultry industry	Professional activity of the backyard owner or member of the family home in connection with poultry industry **	17 (12/70)	0.01**	0.01**
Farmer's assistance	Owner giving assistance to a poultry farmer	7 (5/70)	0.99	0.99
Hunting	Owner or member of family home being a hunter	31 (22/70)	0.93	0.42
Backyard holding proximity	According to the owner, close distance to another backyard holding (1km or less)	74 (52/70)	0.99	0.99
Mortality	Abnormal mortality during the three last months	4 (3/70)	0.99	0.99
Ducks' clinical signs	Clinical signs on ducks during last three months	0 (0/70)	—	—
Poultry clinical signs	Clinical signs on poultry during last three months	6 (4/70)	0.99	0.99
Veterinary consultation	Veterinary visit or consultation during the three last months	1 (1/70)	0.26	0.11